Transfer of Genes to Humans: Early Lessons and Obstacles to Success

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Enough information has been gained from clinical trials to allow the conclusion that human gene transfer is feasible, can evoke biologic responses that are relevant to human disease, and can provide important insights into human biology. Adverse events have been uncommon and have been related to the gene delivery strategies, not to the genetic material being transferred. Human gene transfer still faces significant hurdles before it becomes an established therapeutic strategy. However, its accomplishments to date are impressive, and the logic of the potential usefulness of this clinical paradigm continues to be compelling.

Human gene transfer is a clinical strategy in which the genetic repertoire of somatic cells is modified for therapeutic purposes or to help gain understanding of human biology (*1*, *2*). Essentially, gene transfer involves the delivery, to target cells, of an expression cassette made up of one or more genes and the sequences controlling their expression. This can be carried out ex vivo in a procedure in which the cassette is transferred to cells in the laboratory and the modified cells are then administered to the recipient. Alternatively, human gene transfer can be done in vivo, in a procedure in which the expression cassette is transferred directly to cells within an individual. In both strategies, the transfer process is usually aided by a vector that helps deliver the cassette to the intracellular site where it can function appropriately (*1*, *2*).

Once considered a fantasy that would not become reality for generations, human gene transfer moved from feasibility and safety studies in animals to clinical applications more rapidly than expected by even its most ardent supporters (*1*–*3*). It is not the purpose of this review to detail all human protocols that have been proposed, but to use examples from the available information regarding ongoing human trials (*3*) to define the current status of the field.

How Is Human Gene Transfer Carried Out?

The choice of an ex vivo or in vivo strategy and of the vector used to carry the expression cassette is dictated by the clinical target. The vector systems for which data are available from clinical trials (retroviruses, adenoviruses, and plasmid-liposome complexes) transfer expression cassettes through different mechanisms and thus have distinct advantages and disadvantages for different applications (*1*, *2*).

Vectors. Replication-deficient, recombinant retrovirus vectors can accommodate up to 9 kb of exogenous information (Fig. 1). Retroviruses transfer their genetic information into the genome of the target cell, and thus, theoretically, the target cell's genotype is permanently modified (*1*, *2*, *5*). This is an advantage when treating hereditary and chronic disorders, but it has risks, including the potential for toxicity associated with chronic overexpression or insertional mutagenesis (for example, if the proviral DNA randomly disrupts a tumor suppressor gene or activates an oncogene). The use of retrovirus vectors is limited by the sensitivity of the vector to inactivation, by the fact that target cells must proliferate in order to integrate the proviral DNA into the genome, and by production problems associated with recombination, rearrangements, and low titers (*1*, *2*, *5*). Retrovirus vectors have been used almost entirely in ex vivo gene transfer trials.

Adenovirus vectors in current use accommodate expression cassettes up to 7.5 kb (*1*, *2*, *6*). These vectors enter the cell by means of two receptors: a specific receptor for the adenovirus fiber and $\alpha_v\beta_3$ (or $\alpha_v\beta_5$) surface integrins that serve as a receptor for the adenovirus penton (*7*) (Fig. 2). Adenovirus vectors are well suited for in vivo transfer applications because they can be produced in high titers (up to 10^{13} viral particles/ml) and they efficiently transfer genes to nonreplicating and replicating cells (*8*). The transferred genetic information remains epichromosomal, thus avoiding the risks of permanently altering the cellular genotype or of insertional mutagenesis. However, adenovirus vectors in current use evoke nonspecific inflammation and antivector cellular immunity (*9*). These responses, together with the epichromosomal position of the expression cassette, limit the duration of expression to periods ranging from weeks to months. Thus adenovirus vectors will have to be readministered periodically to maintain their permanent expression. Although it is unlikely that

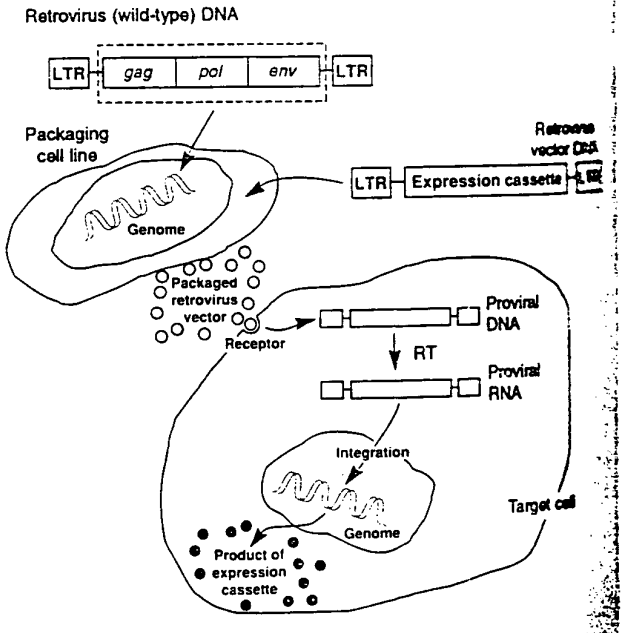

Fig. 1. Retrovirus vector design, production, and gene transfer. Retroviruses are RNA viruses that replicate through a DNA intermediate. The retrovirus vectors administered to humans all use the Maloney murine leukemia virus as the base. The *gag*, *pol*, and *env* sequences are deleted from the virus, rendering it replication-deficient. The expression cassette is inserted, and the infectious replication-deficient retrovirus is produced in a packaging cell line that contains the *gag*, *pol*, and *env* sequences that provide the proteins necessary to package the virus. The vector with its expression cassette enters the target cell via a specific receptor. In the cytoplasm, the reverse transcriptase (RT) carried by the vector converts the vector RNA into the proviral DNA that is randomly integrated into the target cell genome, where the expression cassette makes its product.

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repeat administration will be risky, it is not known whether antibodies directed against vector capsid proteins will limit the efficacy of repetitive administration of these vectors (9). Adenovirus vectors have been used only in in vivo human trials.

In theory, plasmid-liposome complexes have many advantages as gene transfer vectors, in that they can be used to transfer expression cassettes of essentially unlimited size, cannot replicate or recombine to form an infectious agent, and may evoke fewer inflammatory or immune responses because they lack proteins (*10*) (Fig. 3). The disadvantage of these vectors is that they are inefficient, requiring that thousands of plasmids be presented to the target cell in order to achieve successful gene transfer. The available data are not sufficient to determine if repetitive administration of liposomes or DNA poses safety risks. Plasmid-liposome complexes have been used only in in vivo human trials.

Expression cassettes and clinical targets. Human gene transfer studies fall into two categories: marking and therapeutic (Table 1). The marking studies use expression cassettes with bacterial antibiotic-resistant genes, which allow the genetically modified cells to be identified (Table 1). Because the marking genes have no function (other than to permit selection of the modified cells in vitro), the trials using marker genes have been designed to demonstrate the feasibility of human gene transfer, to uncover biologic principles relevant to human disease, and to evaluate safety. These trials have mostly used retrovirus vectors and have focused on malignant disorders or on human immunodeficiency virus (HIV) infection.

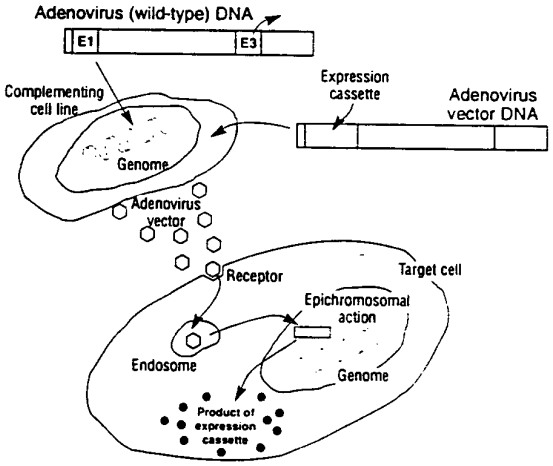

Fig. 2. Adenovirus vector design, production, and gene transfer. Adenoviruses are DNA viruses with a 36-kb genome. The wild-type adenovirus genome is divided into early (E1 to E4) and late (L1 to L5) genes. All adenovirus vectors administered to humans use adenovirus serotypes 2 or 5 as the base. The ability of the adenovirus genome to direct production of adenoviruses is dependent on sequences in E1. To produce an adenovirus vector, the E1 sequences (and E3 sequences if the space is needed) are deleted. The expression cassette is inserted, and the vector DNA is transfected into a complementing cell line with E1 sequences in its genome. The adenovirus vector with its expression cassette is E1$^-$ and thus incapable of replicating. The vector binds to the target cell through an interaction of the adenovirus fiber and penton, each to a specific receptor; moves into a cytoplasmic endosome; and breaks out and delivers its linear, double-stranded DNA genome with the expression cassette into the nucleus, where it functions in an epichromosomal fashion to direct the expression of its product.

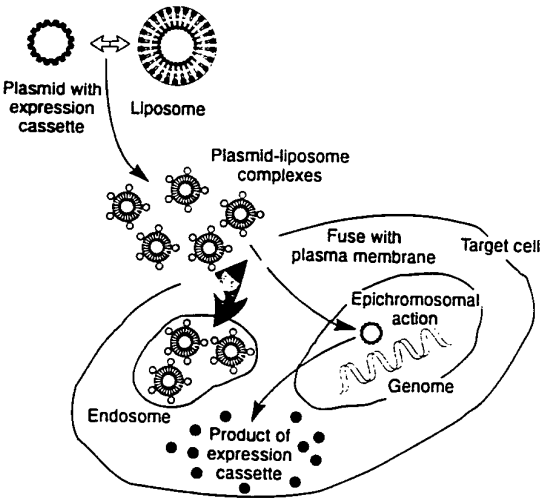

Fig. 3. Plasmid-liposome complex design and gene transfer. The liposomes used in human gene transfer trials have various compositions, but typically include synthetic cationic lipids. The positively charged liposome is complexed to the negatively charged plasmid with its expression cassette. The complexes enter the target cell by fusing with the plasma membrane. The vector does not have an inherent macromolecular structure that conveys information to enable efficient translocation of the plasmid to the nucleus. Consequently, most of the newly introduced genetic material is wasted as it is shunted to cytoplasmic organelles. When used in vivo, it is likely that most, if not all, of the plasmids that reach the nucleus function in an epichromosomal fashion.

The therapeutic trials seek to transfer expression cassettes carrying genes that will evoke biologic responses that are relevant to the treatment of human disease, and to demonstrate that this can be accomplished safely. The therapeutic studies have used retrovirus vectors, adenovirus vectors, or plasmid-liposome complexes. All of the therapeutic trials have been directed toward monogenic hereditary disorders or cancer.

What Has Really Been Accomplished?

Feasibility of gene transfer. Probably the most remarkable conclusion drawn from the human trials is that human gene transfer is indeed feasible. Although gene transfer has not been demonstrated in all recipients, most studies have shown that genes can be transferred to humans whether the strategy is ex vivo or in vivo, and that all vector types function as intended. Taken together, the evidence is overwhelming, with successful human gene transfer having been demonstrated in 28 ex vivo and 10 in vivo studies (Table 1).

In the ex vivo studies with retrovirus vectors, successful gene transfer to humans has been shown by the transfer of marker genes to various classes of T cells (*11–16*), to stem cells in blood and marrow (*16–27*), to tumor-infiltrating lymphocytes (TILs) (*11, 28, 29*), to neoplastic cells of hematopoietic lineage (*16, 17, 20, 21, 25, 26*), and to neoplastic cells derived from solid tumors (Table 1). Although there is variation among ex vivo clinical trials in the proportion of genetically marked cells recovered from the recipients, retroviral vector DNA or marker gene–derived mRNA or both have been observed in cells collected after periods ranging from several weeks to 36 months after administration.

Retrovirus vectors also have been used to transfer therapeutic genes ex vivo, with success demonstrated by the fact that the modified cells exhibit their altered phenotype in vivo for up to 36 months (Table 1). Typically, the expression cassette containing the therapeutic gene also contains an antibiotic-resistance gene, permitting the ex vivo selection of genetically modified cells recovered from the recipient. Successful gene transfer has been demonstrated in cells recovered from children with adenosine deaminase (ADA) deficiency after transfer of the normal ADA complementary DNA (cDNA) to autologous T cells, cord blood, and placental cells (*30–32*); from individuals with solid tumors after transfer of cytokine cDNAs in autologous vaccine strategies to fibroblasts, TILs, or tumor cells (*33–37*); from individuals with familial hypercholesterolemia after transfer of the low-density lipoprotein (LDL) receptor cDNA to autologous hepatocytes (*38, 39*);

from HIV$^+$ siblings after transfer of a chimeric T cell receptor cDNA to blood T cells of a twin (40); and from individuals with tumors who received autologous marrow transplants after transfer of the multidrug resistance 1 cDNA to autologous blood CD34$^+$ stem cells (41). A retrovirus vector has also been used in vivo to successfully transfer a p53 antisense cDNA to lung carcinoma cells (42). Finally, in a combined ex vivo–in vivo strategy for treatment of brain neoplasms, gene transfer to tumor cells has been observed after xenogeneic cells (murine fibroblasts whose genome had been modified with amphotropic packaging sequences) infected with a retrovirus vector containing an expression cassette with the herpes simplex thymidine kinase (HSTK) gene were introduced into the tumor (43).

In in vivo studies with adenovirus vectors, several studies have shown that direct administration of a vector containing the normal human cystic fibrosis transmembrane conductance regulator (CFTR) cDNA to the nasal or bronchial epithelium of individuals with cystic fibrosis (CF) results in transfer of the CFTR cDNA-containing expression cassette to the epithelium, where CFTR mRNA or protein is expressed for at least 9 days (44–50) (Table 1). Direct administration of a plasmid-liposome complex containing an expression cassette with the CFTR cDNA to the nasal epithelium of individuals with CF resulted in expression of CFTR mRNA in the epithelium (51). Finally, plasmid-liposome complexes have

Table 1. Summary of studies showing that transfer of genes to humans is feasible. Data shown are based on published articles and abstracts and on RAC-mandated biannual reports of principal investigators as of the RAC meeting of 8 to 9 June 1995. Abbreviations used for vector study type are RV, retrovirus; Ad, adenovirus; PL, plasmid-liposome complex; M, marker-type study; and T, therapeutic-type study. Abbreviations used for gene products are Neo^R, neomycin phosphotransferase; Hygro, hygromycin phosphotransferase; HSTK, herpes simplex thymidine kinase; ADA, adenosine deaminase; LDLR, low-density lipoprotein receptor; TNF, tumor necrosis factor α; CD4 zeta-R, chimeric T cell receptor; MDR-1, multidrug resistance 1; IL-4, interleukin 4; GM-CSF, granulocyte macrophage colony-stimulating factor; CFTR, cystic fibrosis transmembrane conductance regulator; and B7 + β_2, histocompatibility locus antigen class I–B7 + β_2 microglobulin. Except for Neo^R, Hygro, and HSTK, all genes are cDNAs. Abbreviations used for target cells are TIL, tumor-infiltrating lymphocytes; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus 1; and CTL, cytotoxic T lymphocytes. All target cells are autologous unless otherwise specified. Abbreviations used to characterize study populations are AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphocytic leukemia; ca, carcinoma; and CF, cystic fibrosis. Under in vivo evidence of gene transfer, a plus sign indicates a report of transfer or expression (or both) of an exogenous gene in cells obtained from one or more individuals in the study; time listed is the longest time after administration that gene transfer or expression was observed.

Vector study type	Gene product	Target cells	Study population	In vivo evidence of gene transfer	Principal investigator	Reference number
RV-M	Neo^R	TIL	Melanoma	+ 2 months	Rosenberg, S. A.	(28)
RV-M	Neo^R	TIL	Melanoma	+ 3 months	Lotze, M. T.	(29)
RV-M	Neo^R	Marrow	AML	+36 months	Brenner, M. K.	(16, 17)
RV-M	Neo^R	Marrow	Neuroblastoma	+29 months	Brenner, M. K.	(18)
RV-M	Neo^R	Marrow	Neuroblastoma	+20 months	Brenner, M. K.	(18)
RV-M	Neo^R	Marrow	CML	+ 5 months	Deisseroth, A. B.	(20)
RV-M	Neo^R	Marrow	AML, ALL	+12 months	Cornetta, K.	(21)
RV-M	Neo^R	CD4$^+$, CD8$^+$, blood, TIL	Melanoma, renal cell ca	+	Economou, J. S.	(11)
RV-M	Neo^R	CD34$^+$ blood, marrow	Multiple myeloma	+18 months	Dunbar, C. E.	(22, 23)
RV-M	Neo^R	CD34$^+$ blood, marrow	Breast ca	+18 months	Dunbar, C. E.	(23, 24)
RV-M	Neo^R	Marrow	AML	+12 months	Brenner, M. K.	(25)
RV-M	Neo^R	Normal twin blood T cells†	Identical twins, 1 HIV$^+$	+ 4 months	Walker, R. E.	(12)
RV-M	Neo^R	Blood, marrow	CML	+	Deisseroth, A. B.	(26)
RV-M	Neo^R	CD34$^+$ blood	Metastatic ca, lymphoma	+15 days	Schuening, F. G.	(27)
RV-M	Neo^R	EBV-specific CTL‡	Ca, leukemia	+ 7 months	Heslop, H. E.	(14, 15)
RV-M	Hygro + HSTK	CD8$^+$ HIV *gag* specific, CTL§	HIV$^+$, lymphoma	+14 days	Greenberg, P.	(13)
RV-T	ADA	Blood T cells	ADA deficiency	+36 months	Blaese, R. M.	(30, 31)
RV-T	ADA	Cord blood cells	ADA deficiency	+18 months	Blaese, R. M.	(30, 32)
RV-T	LDLR	Hepatocytes	Familial hypercholesterolemia	+ 4 months	Wilson, J. M.	(38, 39)
RV-T	TNF	TIL	Melanoma	+	Rosenberg, S. A.	(33)
RV-T	IL-2	Tumor cells‖	Metastatic ca	+	Rosenberg, S. A.	(36)
RV-T	IL-2	Neuroblastoma‖	Metastatic ca	+	Brenner, M. K.	(35)
RV-T	CD4 zeta-R	Normal twin blood T cells†	Identical twins, 1 HIV$^+$	+ 4 months	Walker, R. E.	(40)
RV-T	MDR-1	Blood CD34$^+$	Breast ca	+	Deisseroth, A. B.	(41)
RV-T	IL-4	Fibroblasts¶	Metastatic ca	+	Lotze, M. T.	(34)
RV-T	GM-CSF	Melanoma‖	Melanoma	+	Dranoff, G.	(37)
RV-T	Anti-sense p53	Lung ca	Lung ca	+ 1 days	Roth, J. A.	(42)
RV-T*	HSTK	Tumor cells	Glioblastoma	+	Oldfield, E. H.	(43)
Ad-T	CFTR	Nasal, airway epithelium	CF	+ 9 days=	Crystal, R. G.	(44, 45)
Ad-T	CFTR	Nasal epithelium	CF	+	Welsh, M. J.	(46, 47)
Ad-T	CFTR	Nasal epithelium	CF	+	Welsh, M. J.	(48)
Ad-T	CFTR	Airway epithelium	CF	+ 5 days**	Wilson, J. M.	(49)
Ad-T	CFTR	Nasal epithelium	CF	+	Boucher, R. C.	(50)
PL-T	CFTR	Nasal epithelium	CF	+ 4 days	Geddes, D. M.	(51)
PL-T	B7 + β_2	Melanoma	Metastatic ca	+ 3 days	Nabel, G. J.	(52)
PL-T	B7 + β_2	Colorectal ca	Metastatic ca	+	Rubin, J. T.††	(53, 54)
PL-T	B7 + β_2	Renal cell ca	Metastatic ca	+	Volgelzang, N.††	(54, 55)
PL-T	B7 + β_2	Melanoma	Metastatic ca	+	Hersh, E.††	(56)

*This study used a mixed ex vivo–in vivo strategy, in which a xenogenic fibroblast cell line was modified with a retrovirus to produce an amphotropic retrovirus vector containing an expression cassette with the genes for Neo^R + HSTK, and the modified retrovirus-producing cell line was administered directly into the tumor. †Blood T cells from a normal identical twin modified with an expression cassette and then administered to an HIV$^+$ twin. ‡Allogeneic. §The HSTK gene used as a marker gene. ‖Autologous tumor cells modified with an expression cassette, lethally irradiated, and then administered as a "vaccine." ¶Autologous fibroblasts modified with an expression cassette, lethally irradiated, and then administered together with autologous, unmodified tumor cells as a "vaccine." =Messenger RNA at 9 days, vector DNA at 15 days. **A few + cells were observed at 90 days. ††Collaborative study, different institutions.

been used to transfer the human leukocyte antigen (HLA)–B7 and β2 microglobulin cDNAs directly to solid tumors in vivo, with consequent expression of the transfer cassette being seen in the tumor (52–56).

Relevant biologic responses. No human disease has been cured by human gene transfer, and it is not clear when this will be accomplished. However, several studies have demonstrated that therapeutic genes transferred to humans by means of retrovirus, adenovirus, and plasmid-liposome vectors can evoke biologic responses that are relevant to the gene product and to the specific disease state of the recipient (Table 2). Most of the studies demonstrating biologic efficacy have focused on monogenic hereditary disorders, where it is rational to believe that, if the normal gene product could be appropriately expressed at the relevant site, the abnormal biologic phenotype could be corrected.

Severe combined immunodeficiency–ADA deficiency is a fatal recessive disorder caused by mutations in the gene encoding ADA; these mutations cause accumulation of adenosine and 2′-deoxyadenosine, which are toxic to lymphocytes (57). Affected children are unable to generate normal immune responses and develop life-threatening infections. The normal ADA cDNA was transferred ex vivo with a retrovirus vector into T lymphocytes of two children with this disorder, and the modified T cells were expanded in the laboratory and periodically infused into the autologous recipients (30, 31). This resulted in an increase in T cell numbers and in the ADA levels in circulating T cells. The two children now have partially reconstituted immune function, as demonstrated by T cell cytokine release, cytotoxic T cell activity, isohemagglutinin production, and skin test responses to common antigens. In addition, three infants with ADA deficiency who received autologous infusions of cord blood CD34$^+$ stem cells modified ex vivo with a retrovirus vector containing the normal ADA cDNA have also shown evidence of increased numbers of blood T cells and increased ADA levels in T cells (30, 32). The results of the ADA studies are difficult to interpret, because none of these trials have been controlled and the recipients have also received the standard therapy of enzyme infusions with mono-methoxypolyethylene glycol-bovine ADA. Despite these caveats, these observations are consistent with the conclusion that this ex vivo gene transfer strategy evokes biologic responses that are relevant to treatment of ADA deficiency.

Familial hypercholesterolemia is a fatal disorder caused by a deficiency of LDL receptors in the liver that are secondary to mutations in the LDL receptor genes (38, 39, 58). The consequences are high levels of serum cholesterol and LDL cholesterol, premature atherosclerosis, and myocardial infarction. A retrovirus vector was used ex vivo to transfer the normal LDL receptor cDNA to autologous hepatocytes obtained by partial liver resection of an individual with this disorder (38, 39). After reinfusion of the modified hepatocytes into the liver via the portal vein, there was a reduction in LDL cholesterol and in the ratio of LDL to high-density lipoprotein over 18 months, which is consistent with the concept that the corrected cells functioned in vivo to internalize and metabolize LDL cholesterol appropriately. Like the ADA deficiency studies, this study was partially compromised because other therapies were being administered. Furthermore, the LDL receptor gene mutations were mild and could have responded to experimental variables other than the transferred gene (58). However, similar transfer of autologous hepatocytes modified ex vivo to other individuals with more severe mutations of the LDL receptor gene demonstrated partial correction of a variety of lipoprotein-related metabolic parameters, which is consistent with the conclusion that this gene transfer strategy did evoke a relevant response (38).

Cystic fibrosis is the most common lethal hereditary disorder in North America (59). It is caused by mutations in the CFTR gene, a gene coding for an adenosine 3′,5′-monophosphate (cAMP)–regulatable chloride channel in the apical epithelium. As a result of these mutations, the airway epithelium is deficient in CFTR function. This leads to chronic airway infection and inflammation and progressive respiratory derangement. There is compelling logic to the argument that these lung derangements could be prevented if CFTR function could be restored in these cells (60). It is difficult to assess CFTR function in the airway epithelium in vivo in humans, but the nasal

Table 2. Data from human gene transfer studies in which transfer of genetic material has evoked a biologic response that is relevant to the underlying disease.

Disease category	Disease	Strategy	Vector	Gene product*	Target cells	Relevant biologic response	Principal investigator	Reference number
Hereditary	ADA deficiency	Ex vivo	Retrovirus	ADA	Blood T cells and cord blood CD34$^+$ stem cells	Partial restoration of immune response	Blaese, R. M.	(30–32)
	Familial hypercholesterolemia	Ex vivo	Retrovirus	LDLR	Hepatocytes	Partial correction of lipid abnormalities	Wilson, J. M.	(38, 39)
	Cystic fibrosis	In vivo	Adenovirus	CFTR	Nasal epithelium	Partial correction of potential difference abnormalities across the nasal epithelium	Welsh, M. J. Crystal, R. G.	(46, 47) (44, 62)
	Cystic fibrosis	In vivo	Plasmid-liposome complex	CFTR	Nasal epithelium	Partial correction of potential difference abnormalities across the nasal epithelium	Geddes, D. M.	(51)
Acquired	Solid tumors	In vivo	Plasmid-liposome complex	HLA-B7 + β_2	Tumor cells†	Specific immune response to tumor	Nable, G. J. Rubin, J. Vogelzang, N. Hersh, E.	(52) (53, 54) (54, 55) (54, 56)
		Ex vivo	Retrovirus	IL-4	Fibroblasts‡§	Specific and nonspecific immune response to tumor	Lotze, M.	(34)
		Ex vivo	Retrovirus	IL-2	Neuroblastoma‡	Specific and nonspecific immune response to tumor	Brenner, M. K.	(35)

*ADA, adenosine deaminase deficiency; LDLR, low-density lipoprotein receptor; CFTR, cystic fibrosis transmembrane conductance regulator; HLA-B7 + β_2, histocompatibility locus antigen class I–B7 + β_2 microglobulin; IL-4, interleukin-4. †Direct administration to melanoma, colorectal carcinoma, or renal cell carcinoma. ‡Lethally irradiated, used as a "vaccine." §Combined with lethally irradiated, unmodified autologous tumor cells.

epithelium has been used as a surrogate to test the hypothesis that in vivo transfer of the normal CFTR cDNA will correct the functional consequences of CFTR deficiency (*47*, *61*). The parameters measured relate to the observation that the deficiency in CFTR causes an abnormal potential difference between the nasal epithelial surface and subcutaneous tissues. Although the nasal epithelium is not identical to the airway epithelium, two of three studies with adenovirus vectors (*44–47*, *50*, *62*) and one with plasmid-liposome complexes (*51*) have demonstrated that in vivo transfer of the CFTR cDNA to the nasal epithelium evokes a partial correction of these potential difference abnormalities for 1 to 2 weeks.

There are also studies in which human gene transfer appears to have initiated biologic responses that are relevant to therapy for an acquired disorder. These are all "tumor vaccine" studies, based on the hypothesis that exaggerated local expression of an immune-related cytokine might help activate the immune system sufficiently to recognize tumor antigens and control the growth of tumor cells. In one ex vivo study, a retrovirus vector was used to transfer the interleukin-4 (IL-4) cDNA to autologous fibroblasts (*34*). The cells were then irradiated and implanted subcutaneously in the donor together with irradiated, unmodified, autologous tumor cells. In some recipients, this evoked infiltration with $CD3^+$ T cells and tumor-specific $CD4^+$ T cells at the immunization site, as well as enhanced expression of cell adhesion molecules on capillary endothelium. In another trial, autologous neuroblastoma cells modified ex vivo with a retrovirus to contain the IL-2 cDNA were lethally irradiated and implanted subcutaneously (*35*). In some individuals, this evoked systemic augmentation of $CD16^+$ natural killer cells and tumor-specific $CD8^+$ cytotoxic T cells and eosinophilia. Finally, in four trials, in vivo plasmid-liposome complexes were used to transfer a heterologous HLA class I–B7 cDNA and the β_2 microglobulin cDNA directly to solid tumors (*52–56*). In several patients, there was evidence that the gene transfer process initiated amplification of the numbers of detectable, circulating, tumor-specific cytotoxic T cells.

Insights into human biology. Experience with marking studies has shown that human gene transfer can yield important insights into human biology by making it possible to track the fate of genetically marked cells in a recipient. For example, when stored autologous marrow is used to rescue a patient from the suppression of marrow function that complicates high-dose chemotherapy for late-stage malignancy, the individual may subsequently develop a recurrence of the malignancy. Gene transfer marking studies have helped answer the question of whether the recurrence is secondary to a residual tumor in the patient or is derived from malignant cells contaminating the reinfused banked marrow. Several studies that used an ex vivo strategy with a retrovirus vector to mark marrow cells with a neomycin resistance (neo^R) gene and then reinfused the marked marrow have demonstrated that contamination of the autologous marrow with malignant cells is common (*11*, *16–25*). These observations have led to more attention being focused on purging banked autologous marrow of contaminating neoplastic cells before they are reinfused.

There are a number of strategies being developed for the use of ex vivo gene transfer to protect autologous T cells from infection with the HIV-1. None will work, however, if autologous T cells manipulated in the laboratory and then reinfused into an HIV^+ individual have a short biologic half-life. The life-span of an autologous T cell in HIV^+ individuals has been evaluated in identical twin pairs in which one twin is HIV^+ and the other is HIV^- (*12*). A retrovirus vector was used ex vivo to transfer the neo^R gene into the T cells from the normal twin, and the genetically marked cells were then reinfused into the HIV^+ twin. Some $CD4^+$ and $CD8^+$ marked T cells (or their progeny) survived for at least 10 months, providing a baseline to allow future studies to compare the fate of T cells that have been genetically modified to prevent HIV infection.

In a strategy to prevent reactivation of Epstein-Barr virus (EBV) and the accompanying associated lymphoproliferative disease after bone marrow transplantation, allogenic EBV-specific cytotoxic T cells (CTL) were genetically marked with a retrovirus vector, and the cells were infused into individuals at risk (*15*, *16*). This preliminary study suggested that EBV-specific allogenic cells may help control EBV-associated complications of marrow transplantation, and the use of the marker genes demonstrated that the infused EBV-specific CTL persisted in the recipients for 10 weeks.

Two types of therapeutic studies support the biologic concept that minimal correction of a genotype can have significant phenotypic consequences. In the ex vivo study of retrovirus-mediated transfer of the LDL receptor cDNA into autologous hepatocytes in patients with familial hypercholesterolemia, liver biopsy several months after reinfusion of the modified hepatocytes showed that at most 5% of the total hepatocyte population expressed the normal gene in vivo (*38*, *39*, *62*). Despite this minimal correction, in some of the recipients there were changes in LDL-related parameters that suggested LDL receptor function in the liver had been partially restored. Partial phenotypic correction has also been observed in most of the trials of adenovirus- and plasmid-liposome complex–mediated in vivo transfer of the CFTR cDNA to the nasal epithelium in CF, even though the amount of gene transfer and expression has been limited to a small fraction of the target cells (*44–47*, *50*, *51*, *62*).

Finally, when adenovirus vectors are administered to experimental animals, the animals quickly develop circulating neutralizing antibodies directed against the vector (*9*). In two studies of administration of adenovirus vectors to the airways of individuals with CF, no circulating neutralizing antibodies were detected (*44*, *45*, *49*). This is an important observation, because the expression cassette delivered by adenovirus vectors remains epichromosomal, and thus the vector will have to be readministered as its expression wanes. Although it is possible that there are local antibodies to the vectors in these individuals (*9*), the lack of a systemic immune response to such an antigen load is encouraging in that it suggests that antibodies to vectors may not be a major factor limiting persistent vector expression in humans when the lung is repeatedly dosed (*64*).

Safety of gene transfer. The theoretical safety concerns regarding human gene transfer are not trivial. For the individual recipient, there is the possibility of vector-induced inflammation and immune responses, of complementation of replication-deficient vectors leading to overwhelming viral infection, and (for the retrovirus vectors) of insertional mutagenesis. There are also theoretical issues that are important to society, including concerns about modifying the human germ line and about protecting the environment from new infectious agents generated from gene transfer vectors carrying expression cassettes with powerful biologic functions.

There have been adverse events in the human gene transfer trials, including inflammation induced by airway administration of adenovirus vectors (*44–50*, *65*) and by administration to the central nervous system of a xenogenic producer cell line releasing a retrovirus vector (*43*, *66*). However, compared with the total numbers of individuals undergoing gene transfer, adverse events have been rare and have been related mostly to the dose and the manner in which the vectors were administered. Shedding of viral vectors in the in vivo trials was very uncommon and was limited in extent and time (*42*, *44–50*, *65*). No novel infectious agents generated from recombination of the transferred genome and the host genome or other genetic information have been detected, nor has any replication-competent virus related to the vector. Cells modified ex vivo with retrovirus vectors have been infused repeti-

tively without adverse effects (*13*, *30*, *31*, *35*), adenovirus vectors have been administered repetitively in vivo to the nasal (*48*) and bronchial epithelium safely (*64*, *67*), and plasmid-liposome complexes have been administered repetitively to tumors in vivo without complications (*52–56*). Finally, human gene transfer has not been implicated in initiating malignancy, although the numbers of recipients and time of observation will have to be much greater to allow definitive conclusions regarding this issue.

What Are the Obstacles to Successful Human Gene Transfer?

With the successes of the human gene transfer trials have come the sobering realities of the drug development process. Some of the problems are generic for the field, and some are specific for each vector.

Inconsistent results. All of the human gene transfer studies have been plagued by inconsistent results, the bases of which are unclear. For example, in the two children with ADA deficiency receiving intermittent infusions of autologous T cells modified ex vivo with the normal ADA cDNA, the resulting proportion of ADA^+ circulating T cells has varied from 0.1 to 60% (*30*, *31*). In the CF trials, there is evidence that adenovirus vectors and plasmid-liposome complexes can transfer the normal CFTR cDNA to the respiratory epithelium, but expression is observed in at most 5% of the target cells and is not seen in all recipients (*44–51*, *65*). Further, an appropriate biologic response to gene transfer (correction of the abnormal potential difference across the nasal epithelium) has been observed in some patients in most, but not all, of the studies of CFTR cDNA transfer (*44–47*, *50*, *51*, *62*). In most of the ex vivo marrow-marking trials, successful gene transfer is observed intermittently (Table 1).

Humans are not simply large mice. There have been several surprise examples, in which predictions from gene transfer studies in experimental animals have not been borne out in human safety and efficacy trials. In tumor vaccine studies intended to evoke a tumor-directed immune response, there is no convincing evidence (other than anecdotal case reports) that tumors regress, despite the promising observations in experimental animals (*34*, *37*, *52–56*). It has also become apparent that studies in experimental animals may not necessarily predict the toxicology of vectors in humans. In one patient with CF in whom 2×10^9 plaque-forming units of an adenovirus vector containing the CFTR cDNA were administered to the lung, a transient local and systemic inflammatory syndrome was evoked, despite the fact that no clinically apparent toxicity was observed in rodents and nonhuman primates receiving 1000-fold greater doses by the same route (*45*). Likewise, in an ex vivo–in vivo strategy to treat glioblastoma, transfer of xenogenic retrovirus-producing cells to the tumor was accomplished without significant adverse effects in experimental animals, but the human studies have been associated with central nervous system toxicity related to transfer of the cell line to the tumor (*43*, *66*).

Production problems. There are significant hurdles in vector production that must be overcome before large clinical trials can be initiated. Generation of replication-competent virus is observed in production of clinical-grade retrovirus and adenovirus vectors; and lack of reproducibility, aggregation, and contamination with endotoxin complicate the production of clinical-grade plasmid-liposome complexes (*68*).

The perfect vector. The ideal gene transfer vector would be capable of efficiently delivering an expression cassette carrying one or more genes of the size needed for the clinical application. The vector would be specific for its target, not recognized by the immune system, stable and easy to reproducibly produce, and could be purified in large quantities at high concentrations. It would not induce inflammation and would be safe for the recipient and the environment. Finally, it would express the gene (or genes) it carries for as long as required in an appropriately regulated fashion (*69*).

This ideal vector is conceptually impractical, because the human applications of gene transfer are broad, and the ideal vector will likely be different for each application. Clinical experience to date suggests that retrovirus, adenovirus, and plasmid-liposome vectors all need refinement, but each is relatively well suited for the clinical targets at which they have been directed. Further, the technology is now available to create designer vectors that can be optimized for each application. Among the design hurdles for all vectors are the need to increase the efficiency of gene transfer, to increase target specificity and to enable the transferred gene to be regulated. Reproducible production of large amounts of pure vector is a hurdle for all classes of vectors. Some of the vector-specific hurdles are reduction of the risk for insertional mutagenesis in retrovirus vectors, minimization of the amount of immunity and inflammation evoked by the adenovirus vectors, and enhancement of the translocation of the gene to the nucleus for the plasmid-liposome complexes.

There is considerable interest in developing new vectors, but there is controversy as to which vector class is most likely to succeed, particularly for use in in vivo applications. There are two philosophical camps in vector design: viral and nonviral. The viral proponents believe that the most efficient means to deliver an expression cassette in vivo is to package it in a replication-deficient recombinant virus. The logic supporting this approach is the knowledge that viruses are masterful at reproducing themselves, and thus have evolved strategies to efficiently express their genetic information in the cells they infect. The nonviral proponents concede this argument but believe that the redundant anti-immune and inflammatory host defenses against viruses may be a risk to recipients, will limit the duration of expression as the infected cells are recognized by the immune system, and may hinder the efficacy of repeat administration of the vectors. Thus, nonviral vector aficionados believe it is rational to start from scratch to design safe, efficient, gene transfer strategies. In contrast, the viral camp believes that it is best to start with something that works but then to circumvent the replication, immune, and inflammation risks inherent in their use by appropriate vector design. It is most likely that these philosophical differences will eventually disappear as new classes of vectors are designed that incorporate features of viral and nonviral vectors, as dictated by specific clinical applications.

Future Prospects

None of the drug development problems facing human gene transfer are insurmountable, but each will take time to solve. However, the logic underlying the potential usefulness of human gene transfer is compelling; and put in a context in which the human genome project will provide 80,000 to 100,000 human genes that could be used in expression cassettes for human gene transfer, the potential impact of this technology for innovative therapies and increased understanding of human biology is enormous.

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stitutes of Health, Suite 323, 6006 Executive Boulevard, MSC 7052, Bethesda, MD 20892-7052, USA. Human data in this review are derived from published articles and abstracts and from the December 1994 and June 1995 RAC investigator reports. Because the RAC reports are mandated, frequently updated, and public, they are an accurate gauge of the status of the field, although they are not peer-reviewed. Since the first human trial was begun in 1989, there has been an explosion of interest in human gene transfer. In the United States alone, more than 100 human gene transfer protocols have been approved by the RAC, and 697 individuals have participated in human gene transfer trials under RAC-approved protocols (summary data, *RAC Report*, June 1995).
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The Nematode *Caenorhabditis elegans* and Its Genome

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Over the past two decades, the small soil nematode *Caenorhabditis elegans* has becom established as a major model system for the study of a great variety of problems in biolog and medicine. One of its most significant advantages is its simplicity, both in anatomy an in genomic organization. The entire haploid genetic content amounts to 100 million bas pairs of DNA, about 1/30 the size of the human value. As a result, *C. elegans* has als provided a pilot system for the construction of physical maps of larger animal and plar genomes, and subsequently for the complete sequencing of those genomes. By mic 1995, approximately one-fifth of the complete DNA sequence of this animal had bee determined. *Caenorhabditis elegans* provides a test bed not only for the development an application of mapping and sequencing technologies, but also for the interpretation an use of complete sequence information. This article reviews the progress so far toward realizable goal—the total description of the genome of a simple animal.

Caenorhabditis elegans has many attractive features as an experimental system (*1*). The life cycle is simple and rapid, with a 3-day generation time, and populations can be grown with ease on agar plates or in liquid, usually by using *Escherichia coli* as a food source. These populations normally consist of only self-fertilizing hermaphrodites, but cross-fertilization is also possible, with the male sexual form. The option of reproduction by either selfing or crossing leads to very convenient genetics so that mutants can readily be generated, propagated, and analyzed (*2*). A simple freezing protocol pe mits stable storage of all strains, which n tain viability indefinitely in the frozen stat

The animal, about 1 mm long when ful grown, is completely transparent at all sta es of development. Both development ar anatomy are essentially invariant amor wild-type individuals. At maturity, all adu hermaphrodites contain 959 somatic nucl and fewer than 2000 germ cell nuclei. D spite its low cell number, *C. elegans* has ful differentiated tissues corresponding to tho of more complicated animals. The transpa ency and rapid development allow dire examination of cell division and differen ation in living animals with Nomarski m croscopy. The small size of the animal al permits reconstruction of the entire anat my at the ultrastructural level with seri section electron microscopy. However, t

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